

1/3

CTGCAGTCTATTGGATGAAGAGTGTACATATTCATATAATTCTTAAAGTAGGCAGAAATTAAAG GGGATGGAAATATACTTGTACTGCCTTAGATAGTCACCAGGATGTTGTTACAGTCTTCGTTT GAAGAAACAAGGTSAAGCCATCTGCTTAACTTATGTCCACATTCTCTCAAGAGCATTGTCCTA TTTGTAGAATTATCTATATTGTTAAGAATCATCTCCATTGTTAAGATTTTGTGGGCTGGAGATC CAGCTCTGTTGATAAAGTGCTTGCCTAACATGCATGAAGTCCTAGGTTCTATTCCCAAGGCTAC ATAAAACCTTGTGTTGTGATGAATGCCTGTAATCCCAGTACGCAGCAAGGAGAGACAAGGAGGA TCAGAAGCTTAAGGACATCATTTTGTACATAGTGAGTTTGAGGAAAGCTGAGGTTACATGGAAC ATCATAAACATTTAACTCATTGATTATGTTGAGAGTTGTCCCTCAAGAACCAATGGCCAAAC ATCCACTGAGGATACACGGAAGCTTAGAAAATCTCTAATTAAAATCCTGACATAATGGAAGTGC TCACAAACCAGCCAACACCTAATAAAACCAGTGGCAAGAGCAACAACTCGGCATTTTTCTACTT TGAATCCTGCCAACCCCCTTTTCTAGCCATACTCTTGCTACTCATAGCATATACTGTGATCCTA CATCCCTTTTACGGTCATCTACACTCTGATGGACCACTGGGTATTTGGGAACACTATGTGTAAA CTCACTTCCTACGTGCAAAGTGTCTCAGTTTCTGTGTCCATATTCTCCCTTGTGTTGATTGCTA TTGAACGATATCAGCTGATTGTGAACCCCCGTGGCTGGAAACCCAGAGTAGCTCATGCCTATTG GGGGATCATCTTGATTTGGCTCATTTCTCTGACATTGTCTATTCCCTTATTCCTGTCCTACCAC GTATTTTGTCCCTCTGGGTTTCATTCTTATCTGCTACCTGAAGATCGTTCTCTGCCTCCGAAAA AGAACTAGGCAGGTGGACAGGAGAAAGGAAAATAAGAGCCGTCTCAATGAGAACAAGAGGGTAA ATGTGATGTTGATTTCCATCGTAGTGACTTTTGGAGCCTGCTGGTTGCCCCTTGAACATTTTCAA TGTCATCTTCGACTGGTATCATGAGATGCTGATGAGCTGCCACCACGACCTGGTATTTGTAGTT TGCCACTTGATTGCTATGGTTTCTACTTGCATAAATCCTCTCTTTTATGGATTTCTCAACAAAA ACTTCCAGAAGGATCTAATGATGCTTATTCACCACTGTTGGTGTGGTGAACCTCAGGAAAGTTA TGAAAATATTGCCATGTCTACTATGCACACAGATGAATCCAAGGGATCATTAAAACTGGCTCAC AGAAATAGTATGGAATAGGGCAAGGTGCAGAGGAAGCCAGACTTAAACACATAATATCTTTGGG CCCAGTTTTGCTTTAAGTTAAGCATGTCTACTCCATTCAGCCATAGAACACACAGAGATTTATC CCTACCCTTTCTTTTTTCCTTTGGAAGAATAATAACTTAAACAACCTAGACATCATTACTGAG GAAGAGAACAAAAATGAGAGGACATACAAGGACAGCAGAGATGTCTGGGGTACAAAATTCACGT TATTCGCTGGAATAGCTAGAAAGTTATTAGTTGTGCTGCAG (SEQ ID NO:1)

FIGURE 1

underlined = deleted in targeting construct

[] = sequence flanking Neo insert in targeting construct

[CTGCAGTCTATTGGATGAAGAGTGTACATATTCATATAATTCTTAAAGTAGGCAGAAAT TAAAGGGGATGGAAATATATACTTGTACTGCCTTAGATAGTCACCAGGATGTTGTTACAG GAGAGAGAGAGAAGAAGAAAACAAGGTSAAGCCATCTGCTTAACTTATGTCCACAT TCTCTCAAGAGCATTGTCCTATTTGTAGAATTATCTATATTGTTAAGAATCATCTCCATT GTTAAGATTTTGTGGGCTGGAGATCCAGCTCTGTTGATAAAGTGCTTGCCTAACATGCAT ${\tt GAAGTCCTAGGTTCTATTCCCAAGGCTACATAAAACCTTGTGTTGTGATGAATGCCTGTA}$ ATCCCAGTACGCAGCAAGGAGACAAGGAGGATCAGAAGCTTAAGGACATCATTTTGTA AAAACAAAACAAAACCTTCTACTAATATTCTGGATTCTGTTTGATTTTTAGGATCTCAAG CTCATTGATTATGTTGAGAGTTGTCCCTCAAGAACCAATGGCCAAACATCCACTGAGG ATACACGGAAGCTTAGAAAATCTCTAATTAAAATCCTGACATAATGGAAGTGCTCACAAA CCAGCC] AACACCTAATAAAACCAGTGGCAAGAGCAACAACTCGGCATTTTTCTACTTTG AATCCTGCCAACCCCCTTTTCTAGCCATACTCTTGCTACTCATAGCATATACTGTGATCC <u>TAATCATGGGCATTTTTGGAAACCTCTCTCTTATCATCATCATCTTTAAGAAACAGAGAG</u> AAGCTCAAAATGTTACCAACATACTGATT [GCCAACCTGTCCCTCTCTGACATCTTGGTG TGTGTCATGTGCATCCCTTTTACGGTCATCTACACTCTGATGGACCACTGGGTATTTGGG AACACTATGTGTAAACTCACTTCCTACGTGCAAAGTGTCTCAGTTTCTGTGTCCATATTC TCCCTTGTGTTGATTGCTATTGAACGATATCAGCTGATTGTGAACCCCCGTGGCTGGAAA $\tt CCCAGAGTAGCTCATGCCTATTGGGGGGATCATCTTGATTTGGCTCATTTCTCTGACATTG$ CCTACTGACATCTACACCCACCAGGTAGCTTGTGTGGAGATTTGGCCTTCTAAACTGAAC CAACTCCTCTTTTCTACATCATTATTTATGCTCCAGTATTTTGTCCCTCTGGGTTTCATT CTTATCTGCTACCTGAAGATCGTTCTCTGCCTCCGAAAAAGAACTAGGCAGGTGGACAGG AGAAAGGAAAATAAGAGCCGTCTCAATGAGAACAAGAGGGTAAATGTGATGTTGATTTCC $\tt ATCGTAGTGACTTTTGGAGCCTGCTGGTTGCCCTTGAACATTTTCAATGTCATCTTCGAC$ TGGTATCATGAGATGCTGATGAGCTGCCACCACGACCTGGTATTTGTAGTTTGCCACTTG ATTGCTATGGTTTCTACTTGCATAAATCCTCTCTTTTATGGATTTCTCAACAAAAACTTC CAGAAG] GATCTAATGATGCTTATTCACCACTGTTGGTGTGGTGAACCTCAGGAAAGTTA TGAAAATATTGCCATGTCTACTATGCACACAGATGAATCCAAGGGATCATTAAAACTGGC TCACATACCAACAGGCATATAGAAACTGGTAAGCAAAATCAAAGCCCTTCTGTTATGAAA GAAAGAGAAGAATAGTATGGAATAGGGCAAGGTGCAGAGGAAGCCAGACTTAAACACAT AATATCTTTGGGCCCAGTTTTGCTTTAAGTTAAGCATGTCTACTCCATTCAGCCATAGAA CACACAGAGATTTATCCCTACCCTTTCTTTTTTTCCTTTGGAAGAATAATAACTTAAACA ACCTAGACATCATTACTGAGGAAGAGAACAAAAATGAGAGAGCATACAAGGACAGCAGAG ATGTCTGGGGTACAAAATTCACGTTATTCGCTGGAATAGCTAGAAAGTTATTAGTTGTGC

FIGURE 2A

Gene Sequence Structure 846 bp Sequence Deleted 1047 bp Size of CDS: 2281 bp Targeting Vector* LacZ-Neo (genomic sequence) Cassette 3' arm 5' arm Construct Number: 2762 5' probe Arm Length: 5': 5 kb 3': 0.5 kb **5'**>TTTAGGATCTCAAGAGCATGC 5'>GCCAACCTGTCCCTCTCTGAC TGACGTCATTTATGTGTTTCCATC ATCTTGGTGTGTGTCATGTGCATC AGATACTGACAGAGATCATAAACA CCTTTTACGGTCATCTACACTCTG TTTAACTCATTGATTATATGTTGA ATGGACCACTGGGTATTTGGGAAC GAGTTGTCCCTCAAGAACCAATGG ACTATGTGTAAACTCACTTCCTAC **Targeting Vector** CCAAACATCCACTGAGGATACACG GTGCAAAGTGTCTCAGTTTCTGTG - Endogenous Locus GAAGCTTAGAAAATCTCTAATTAA ${\tt TCCATATTCTCCCTTGTGTTGATT}$ AATCCTGACATAATGGAAGTGCTC GCTATTGAACGATATCAGCTGATT * Not drawn to scale ACAAACCAGCC<3' GTGAACCCCCG<3' (SEQ ID NO:2) (SEQ ID NO:3)